SHORTER ARTICLES AND DISCUSSION

ON THE RESISTANCE OF FUNDULUS TO CONCENTRATED SEA WATER[1]

I. THERE is at Bermuda a Fundulus, described by Günther ('79) under the name *F. bermudæ,* which is very closely related to *F. heteroclitus,* if not indeed specifically identical with it.[2] The common habitat of this Fundulus is along the shores of mangrove swamps, in water normally having a salinity of 35–36 per mille (Cl = 20 ± per mille; sp. gr. about $1.0225^{27}/_{4}°$). When this Fundulus was placed in sea water which was allowed to evaporate at laboratory temperature (about 27°) a good number of specimens were found to resist a concentration of about $^{15}/_{8}$ *M* sea water (Cl = 67 per mille). According to Loeb ('13, '16), *F. heteroclitus* at Woods Hole may be brought to live in a concentration equivalent to $^{10}/_{8}$ *M* or $^{11}/_{8}$ *M,* if the water be slowly evaporated, but a $^{12}/_{8}$ *M* concentration is rapidly fatal. Sea water at Woods Hole is at about *M*/2 (salinity = 32 per mille ±), with a freezing-point depression of 1.81° (Scott, '13), whereas the Bermuda sea water is nearly $^{9}/_{16}$ *M,* with (according to Knudsen's Table, 5) a freezing-point depression of 1.95.° McClendon ('11) found the Δ of Tortugas water (S = 36 per mille ±) to be 2.03°.

Is the considerable difference noted in the resistance of *Fundulus* taken from these differing environments to be regarded as an instance of adaptation brought about in nature?

II. Tests were made at different seasons to discover the upper limit of concentration which the Bermuda fundulus will tolerate. One of these experiments may be cited as an example:

Experiment 4.—Aug. 20, 1917. Six fundulus were placed in each of three glass aquaria containing 2 liters of sea water (Cl = 19.65°/$_{00}$; S = 35.50°/$_{00}$) brought from the mangrove creek in which the fundulus were collected. The water was allowed to evaporate at room temperature (28°). In aquarium No. 1, two fishes were still alive on Sept. 7,

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[2] Some of the specimens used in these experiments were examined by Mr. Samuel Garman, of the Museum of Comparative Zoology, who pronounces them to be *Fundulus heteroclitus,* var. *bermudæ* Goode and Bean.

at which time the water was so concentrated that the salinity titration (of an aliquot part of a dilution with distilled water) gave Cl = 66.69 $^0/_{00}$. These two fishes lived until Sept. 11, when the Cl content of the water was 72 $^0/_{00}$. Similarly, in the other two aquaria the maximal concentrations were Cl = 66.83, 66.90 $^0/_{00}$.

Other tests gave comparable results, about one third of the fundulus living until the Cl content of the water was nearly 67 per mille (= $^{15}/_8$ *M* sea water). Provided the process of evaporation occupied at least two weeks, slowing the evaporation of the water did not seem to augment the resistance of the fishes. Fundulus "adapted" by slow evaporation lived in $^{14}/_8$ to $^{15}/_8$ *M* solutions for a week or more. When a concentration of about Cl = 70 per mille ($^{16}/_8$ *M*) was reached, the fishes usually died very rapidly, although an occasional one survived until the Cl content was 75 per mille, whereas at Woods Hole, according to Loeb, the rapidly fatal concentration is $^{12}/_8$ *M*. In each case the maximal concentration endured for any length of time is about three times that normally experienced by the fish.

III. While Loeb found the resistance of *F. heteroclitus* to be only slightly enhanced by a series of "adapting" experiences in waters of gradually increasing concentration, it might nevertheless be argued that a more gradual series of changes, leading to normal life in more saline water, would be more efficacious. There are several facts which dispose of this supposition, aside from the somewhat disproportionately great increase in absolute resistance which is exhibited by the Bermuda form.

The Bermuda fundulus is found not only in the mangrove creeks, but also in certain landlocked brackish ponds (*e. g.*, Warwick Pond, Trott's Pond) where the salinity is usually 14.5–23 per mille (Cl = 8.0–12.7 per mille), although it varies somewhat with the rainfall. The level in these ponds rises and falls slightly with the ocean tide, but there is no variation in salinity synchronous with this. Fundulus were taken from these ponds and placed in sea water which was allowed to evaporate slowly, and others were put in pond water which was allowed to evaporate. The lethal concentrations in these two series were practically identical, namely, at about Cl = 65–70 per mille, one third of the individuals usually surviving until the Cl content reached 66–67 per mille, which is essentially the same maximal concentration as that found with the individuals living normally in undiluted sea water. *F. bermudæ* will live

for a long time in rain water containing but a trace of salts, and those from the brackish ponds will live equally well when suddenly transferred to sea water of full salinity (36 per mille).

Now, the fundulus living in the brackish ponds have been there for an indefinitely long period. They reproduce there, and must be regarded as "adapted" to the low salinity of the ponds. There is consequently no reason to expect, on the adaptation hypothesis, that they should be as resistant to concentrated sea water as the individuals living in Fairyland Creek, for example, where the water is of normal salinity. Yet this appears to be the case. It is true that this species inhabits other brackish swamp pools at Bermuda, where the salinity undergoes considerable changes. But if the high resistance of the isolated-pond fundulus were to be explained as the result of a persisting mechanism inherited from ancestors adapted to withstand changes in salinity, then it will be noted that the appeal to adaptation in the first place becomes not merely superfluous, but inconsistent.

IV. There is another explanation available, which probably accounts for the high resistance of the sea-water and brackish-pond fundulus to concentrated solutions. This explanation considers that the conditions of temperature and the composition of the water (especially in the brackish ponds) have shifted the protoplasmic equilibria which determine the composition (and hence the permeability and the resistance) of the limiting membranes of the fish's body.

Loeb and Wasteneys ('12, '15) found that fundulus taken from a temperature of 10° died in the course of several hours when kept at 29°, in a few minutes at 35°; whereas those maintained at 27° would live indefinitely if transferred to 35°. The fundulus at Bermuda living in the mangrove creeks are at a temperature of 26°–27° (during the summer months). They withstood for some hours a temperature of at least 37°, and died when heated to 40.9°. In the shallow landlocked ponds the surface temperature was 30°–33°. Fundulus from these ponds withstood for several hours a temperature of 39°–40°, and died when heated to 42.6°. The upper temperature limit was also determined for fundulus from the brackish ponds which (at 27°) had for two weeks been living in $^{10.5}/_{8}$ *M* sea water; they withstood 40°, and died at 42.5°. Other individuals living for the same period in $^{5}/_{8}$ *M* sea water withstood 40°, and died quickly

at 41.5°. There is thus seen in fundulus the correspondence usually found in every thermal species between the temperature at which the animal lives and the maximal temperature which it can successfully withstand (cf. Mayer, '14).

The alkalinity of the waters inhabited by the Bermuda fundulus is quite various. In the mangrove creeks the reaction of the water along the shore may vary a little with the state of the tide, but is usually not far from $p_H = 8.1$. In the landlocked brackish ponds, however, the alkalinity is commonly much higher than this. In one pond, where many algæ were growing, the alkalinity was conspicuously high, $p_H = 9.0$–9.2 (except after rains);[3] and in another, with a sparser growth of water plants, the reaction usually observed was $p_H = 8.7$. Rain water had at this time a consistent reaction of $p_H = 5.9$–6.0, but after contact with the soil and limestone it quickly becomes alkaline, so that the water in cave pools, or dripping from growing stalactites, was found to have a reaction of $p_H = 7.9$–8.0. The high alkalinity of the pond waters may be important in determining the survival of fundulus in abnormal solutions.

If the idea is correct that the composition (*e. g.*, the calcium content) and (?) the temperature of the sea water or pond water are responsible for the high resistance of the brackish-pond fundulus to concentrated sea water, then we should expect that NaCl solutions would be less toxic for the Bermuda fundulus than for the northern variety, which, according to Loeb, and Wasteneys ('12), is killed by 1 *M* NaCl in less than one half hour (at about 18°–20°, it is inferred). The pure NaCl solution increases the permeability of the surface membranes of fundulus. At 25°–27°, 50 per cent. of the Bermuda fundulus lived forty-five minutes in 1 *M* NaCl solution, when the specimens were taken from the mangrove creeks. Individuals from the landlocked brackish ponds lived about the same length of time (even after rapid washing, three times, in changes of NaCl solution). At 20° they lived a little longer. This result is in agreement with that obtained experimentally by Loeb ('16, p. 332), namely, that fundulus adapted to higher concentrations of sea water became more resistant to pure NaCl solutions; those brought artificially to live in $^{10}/_8$ *M* sea water could live two to

[3] Moore and his co-workers ('14) state that the photosynthetic activities of algæ are capable of increasing the alkalinity of sea water until $p_H = 9.0$; I have been able to confirm this statement through experiments with the green alga *Valonia*.

three days in $\frac{6}{8}$ M NaCl, which killed fundulus taken directly from the sea in less than four hours. But in the present case the fundulus from the brackish ponds lived about equally well in pure NaCl solution. The cause of this behavior, which would not be expected from an adaptational standpoint, is believed to lie in the direct effect of the calcium or some other element of the pond water. In $\frac{6}{8}$ M NaCl solution fundulus from the mangrove creeks and those from a brackish pond lived respectively 3.5 and 4.0 hours, roughly, at 20°. The experiments with 1 M NaCl seemed more valuable for the purposes of this inquiry, because of the more rapid toxic effects, secondary complications being thus more easily avoided.

V. The fact that the Bermuda fundulus, closely related to *F. heteroclitus,* but living usually in water of greater salinity than that inhabited by the Woods Hole variety, seems also able to withstand a distinctly higher concentration of evaporated sea water than the latter will tolerate, is therefore not to be considered an expression of adaptation to life in more saline water. Other members of the same species at Bermuda which are confined to brackish ponds of low salinity, and have for at least several generations been restricted to this environment, are equally resistant to concentrated sea water, and to pure NaCl solutions, and more resistant than the fundulus at Woods Hole, indicating that the resistance of the Bermuda form is due to a direct action of certain constituents of the waters in which it lives upon the composition of its surface membranes.[4]

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[4] The observations here briefly reported in a preliminary way have been temporarily interrupted, but it is hoped to continue them in the near future, as some indication was noted of a lowered resistance to pure NaCl correlated with a decreased alkalinity of the pond-waters, during the winter months. The determinations of alkalinity were made with the aid of apparatus purchased through a grant to the Station from the C. M. Warren Fund of the American Academy of Arts and Sciences.

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A NOTE ON THE FATE OF INDIVIDUALS HOMOZYGOUS FOR CERTAIN COLOR FACTORS IN MICE

Isben and Steigleder have reported on certain breeding experiments with mice which produce evidence in support of the view advanced by Castle and the writer in 1910, and later strengthened by Kirkham, 1917, that homozygous yellow mice were formed but perished during embryonic life.

At the time that they were collecting their data, the writer was, on a smaller scale, carrying on similar experiments. In the course of these experiments, certain data confirmatory to the results of Isben and Steigleder and of Kirkham were obtained. It seems best at this time to put these results on record.

The embryos referred to as "abnormal" may be considered as falling in Isben's and Steigleder's Class A of dead embryos, that is to say, those in which development ceased shortly after implantation as contrasted with those in which death had resulted probably from overcrowding within the uterus during the latter part of the period of gestation.

Three types of matings to control the results in yellow × yellow crosses were made. In all cases, the non-yellow animals used were taken from the same stock as that producing the yellows. The control matings made were as follows: Yellow female × non-yellow male, non-yellow female × yellow male and finally non-yellows crossed *inter se*. The numbers obtained are small and are grouped together in the following table: